A Study of the Carotenoids Echinenone and Myxoxanthin with Special Reference to their Probable Identity

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It has recently been shown (Goodwin & Taha, 1950) that the ketocarotenoid present in the gonads of the limpets *Patella vulgata* and *P. depressa* is identical with the pigments isolated from the gonads of the sea-urchin *Paracentrotus lividus*, and from the blue fresh-water alga *Aphanizomenon flos-aquae*, and termed respectively echinenone (Lederer, 1935) and aphanin (Tischer, 1938, 1939). (In this paper the term echinenone will be used.) It was also suggested that echinenone was probably identical with the carotenoid, myxoxanthin, characteristic of the blue-green algae (Myxophyceae), which was isolated crystalline from the fresh-water alga *Oscillatoria rubescens* (Heilbron & Lythgoe, 1936; Karrer & Rutschmann, 1944). This identity had previously been suggested by Lederer, but considered improbable by Heilbron (see Karrer & Jucker, 1948, p. 335). It has recently been possible to obtain a small amount of myxoxanthin from a mixed culture of *Oscillatoria* spp. and to compare it directly with a sample of echinenone.

EXPERIMENTAL

Extraction of pigments. The sample of mixed *Oscillatoria* spp. was covered with 20% (w/v) ethanolic KOH and allowed to stand for 24 hr. at room temperature, under N_2 and in the dark. The mixture was then filtered through a Büchner funnel and the green filtrate transferred to a separating funnel. After addition of an equal volume of water the carotenoids were removed by extraction with four successive portions of ethyl ether freshly distilled over reduced Fe. The combined ether extracts were washed free from alkali, dried over anhydrous Na_2SO_4 and the ether removed *in vacuo*; the residue was dissolved in a small volume of light petroleum (b.p. 40–60°).

Separation and purification of myxoxanthin. The light petroleum solution was extracted three to four times with an equal volume of 90% (v/v) aqueous methanol and the methanol fractions (the hypophasic pigments) discarded. The petroleum fraction was taken to dryness *in vacuo*, the residue dissolved in a little methanol, and cooled in solid CO_2 . The steroids which crystallized out were removed by filtration through a Büchner funnel packed with solid CO_2 . The methanol was then removed *in vacuo* at about 30° and the purified residue dissolved in light petroleum (b.p. 40–60°) and chromatographed on alumina (Spence, Grade O) deactivated with methanol (Goodwin & Taha, 1950). Two zones were obtained, the upper being brick-red and the lower orange and diffuse; the latter was eluted with light petroleum (b.p. 40–60°) containing 1–2% (v/v) of ethyl

ether, and shown to be β -carotene. The brick-red zone was eluted with ethyl ether and the solvent removed; the residue was dissolved in light petroleum and the fraction rechromatographed. The procedure was repeated twice and the purified product compared chromatographically and spectroscopically with a similarly purified specimen of echinenone prepared from *Patella* gonads.

Derivates of myxoxanthin. Two derivates of myxoxanthin, the oxime and the alcohol, prepared by reduction with aluminium isopropoxide, were prepared in a chromatographically homogeneous state as described by Goodwin & Taha (1950) for echinenone. They were also compared chromatographically and spectroscopically with the corresponding derivates of echinenone. The reduced product was also compared with a chromatographically pure specimen of cryptoxanthin obtained from yellow maize.

RESULTS AND DISCUSSION

Spectrographically, the sample of myxoxanthin obtained in the present investigation was indistinguishable from echinenone; similarly, the corresponding derivatives, the oxime and the alcohol, were also indistinguishable from those of echinenone; the positions of the absorption bands were identical with those previously recorded for echinenone and its derivatives (Goodwin & Taha, 1950) and are not recorded here. For illustration, however, the complete visual absorption curves for myxoxanthin and echinenone are recorded in Fig. 1; it will be seen that they are practically identical over the whole of this spectral range. When compared with echinenone using the mixed chromatogram technique (Goodwin & Taha, 1950), no separation of the two pigments could be obtained; furthermore, no chromatographic separation of mixtures of the oximes or the alcohols could be demonstrated. Considering this evidence, there is, therefore, no reasonable doubt that echinenone and myxoxanthin are identical.

Goodwin & Taha (1950) have shown that spectroscopic evidence indicates 4-keto- β -carotene as the probable structure of echinenone, and at the same time pointed out that, even if myxoxanthin and echinenone were not the same pigment, the structure (I) assigned to myxoxanthin (Heilbron & Lythgoe, 1936; Karrer & Rutschmann, 1944) was not tenable on the spectroscopic evidence; the present results indicate that this decision was justified. One additional point concerning struc-

ture (I) should perhaps be made; this structure was finally accepted on an interpretation of spectral data which cannot now be considered valid. It was assumed that the single-banded

spectrum of this and other ketocarotenoids is due to the simultaneous conjugation of the carbonyl group with two sets of unsaturated linkages (Heilbron & Lythgoe, 1936). It is now known that this

Fig. 1. The absorption spectra of echinenone (---) and myxoxanthin (-.-.-) in light petroleum (b.p. 40–60°). Scales for the extinctions (E) have been so chosen that the ordinates representing $E_{\max}$ for the two pigments are equal (1.0).

is not a necessary condition for the production of a single-banded spectrum, for such a spectrum is exhibited by astaxanthin (Goodwin & Srisukh, 1949)

which is 3:3'-dihydroxy-4:4'-diketo- β -carotene (Kuhn & Sörensen, 1938).

It has been shown in the present work that the Oppenauer-reduction product of echinenone is chromatically indistinguishable from cryptoxanthin. Previously (Goodwin & Taha, 1950), they had only been compared spectroscopically. This provides further evidence in favour of considering cryptoxanthin to be 4-hydroxy- and not 3-hydroxy- β -carotene. There is no compelling reason for accepting 3-hydroxy- β -carotene as the structure, for it is based on an analogy which is not too secure (see Karrer & Jucker, 1948, p. 178). It is only the 3'-hydroxyl group, in the α -ionone ring, which is unequivocally fixed in lutein (generally accepted as 3:3'-dihydroxy- α -carotene; see Karrer & Jucker, 1948, p. 205), the other position (3) in the β -ionone ring, is fixed by analogy; the 3-position in cryptoxanthin is then fixed by analogy with the doubtful position of the hydroxyl group in the β -ionone residue of lutein. This 'analogy of symmetry' cannot be considered to carry a great deal of weight because the lutein molecule is asymmetrical. It is at least as logical to argue that the plant, in synthesizing lutein, would maintain the same relative positions of the double bond and the hydroxyl group in both the α - and β -ionone residues. On this reasoning lutein would then be 3':4-dihydroxy- α -carotene and cryptoxanthin 4-hydroxy- β -carotene. There is thus good reason to believe that 4-hydroxy- β -carotene best represents the structure of cryptoxanthin, but final proof of this depends on extensive degradation studies.

SUMMARY

1. The carotenoid myxoxanthin, its oxime and its Oppenauer-reduction product (alcohol) are spectroscopically and chromatographically indistinguishable from echinenone and its corresponding derivatives; it is therefore concluded that the pigments are identical.

2. The Oppenauer-reduction product of echinenone is spectrographically and chromatographically identical with cryptoxanthin. This strongly suggests that the structure of cryptoxanthin is 4-hydroxy- and not 3-hydroxy- β -carotene.

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